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IN VITRO STUDY AND MICROPROPAGATION OF STRAWBERRY.(*Fragaria x ananassa*)

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Abstract: *The micropropagation of meristem tip of Strawberry resulted in multipal shoots when cultured on MS medium supplemented with different concentration of BA with KIN. The highest i.e. 90% of usable shoots induction where observed at the concentration (1.5mg/l BA with 0.5 mg/l charcoal and PVP). Best initiated shoots (80% and90%) where then use for multiplication and inoculated on MS media supplemented with 1.5mg/l BA and 0.5% mg/l KIN along with as a substituted of KIN two additives i.e. charcoal and PVP where used. Out of these variable treatment1.5mg/l BA and 0.5mg/l charcoal showed maximum shoots i.e. 2.83±0.4 per explants. The elongation of roots where very slow and best rooting response was observed on media fortified with 1.0mg/l BA i.e. 3.5±0.5 number of roots. The problem of phenolic browning was minimized by effective use of activated charcoal. The regenerated plants are under incubation for significant root induction and will be transfer for primary hardening.*

Abbreviations: *BA= Benzyl adenine, KIN= Kinetin, MS= Murashige and Skoog medium*

Key Words: *Benzyl adenine, Kinetin, Murashige and Skoog medium.*

Introduction

Fragaria x ananassa belongs to the member of the kingdom Plantae, order Rosales, family Rosaceae, subfamily Rosoidea and genus *Fragaria* commonly known as Strawberry. It is cultivated all around the world, not only for its digestive and tonic properties but because of the nutritional value of its fruits, important source of folate, vitamin C, fiber, potassium, flavonoids phytochemical and antioxidant.

Fragaria x ananassa is used in processed form such as cooked and sweetened preserve jam or jellies and frozen whole berries or sweetened juices extracts or flowering and variety of other processed products can be made.

Strawberry world production has a significant increase in last 25 years. From1985-2010, land with strawberry cultivation increase by 25%. There are 57 countries contributed to this production, 9 of them produce 72% of total the total production was 43,66,889 tones, the major producer nation is USA with production 12,92,780 tones. Approximate 15% of the world strawberry production is exported as fresh fruit and in USA more than 25% is processed. The major fresh strawberry exporter countries are Spain and USA.Strawberry is also grown in India, Pakistan, Bangladesh and China.

Among the many spp. of strawberry, reproduction occurs both sexually and asexually. In sexually reproduction cross-pollination and self – pollination occurs but depends on species. Strawberry plant produce asexually through the stolons, as known as runner .strawberry tree is difficult to propogate by seed due to genetic variation and specific requirement of see germination. In addition, rooting percentage of cutting is relatively low.

In general strawberry can be managed easily under *in vitro* condition. Meristem tips, generally obtained from runner of virus free plant are commonly used to establish *in vitro* culture. Micropropagation of strawberry by axillary bud has been studied intensively for a long time on a commercial scale. Tissue cultured derived strawberry plant are estimated to cost four to five times more than plants.

Several advantages of micrpropagation strawberrys are such as, ability to multiply virus-free

stock rapidly and in particular the improved capacity of these plants to reduce runner for planting in the field.

The production program suggested by *Vit.et. Al* or commercial planting material is based on the selection of economically important clones; freeing these of virus by a combination of heat therapy and merisetm culture. Tissue culture aimed particularly at the rapid propagation of breeding material.

Hence the main objective of the present study is to find out effect of plant regulators on shoots and root regeneration.

Materials and Method

Plant Material and Explants: Mother plant of strawberry (*Fragaria x ananassa*). Meristem 1-2 cm explants taken mother plant as length was suitable for sterilization procedures. The surface sterilization of explants was carried out by washing the explants under running tap water then explants was treated with Bavistin (w/v) for 10 min then ,further explants was rinsed with distilled water 2-3 times. Then treat the explants with 0.1%HgCl2 (w/v) for a min, treated in laminar air flow. Rinsed the explants 2-3 times with sterile distilled water to remove all traces. Then further explants were treated with antibiotics such as Gentamycin/Streptocyclin (v/v andw/v) and at last sterilized explants were cut into 1-1.5 cm for ignition.

Medium and Culture Conditions: The explants were culture on Murashige and Skoog (MS) medium auxins and cytokines used for this experiment are given below

I) Shoot induction:

Table 1: Hormone concentration for shoot inductuion

Identification No.	Media+BA+KIN in mg /l	No .of Bottle
1	MS+1.4+0.5	10
2	MS+1.5+0.5	10
3	MS+2.0+0.1	10
4	MS+2.0+0.5	10
5	MS+1.5+0.5(Charcoal)	10

II) Root Induction

Table 2: Hormone concentration for root induction

Identification No.	Media+IBA+Charcoal in mg/l	No. of Bottle
1	MS+0.5+500	5
2	MS+1.0+500	5
3	MS+1.5+500	5

Incubation of culture

The culture after inoculation were transfer in culture room. The culture room conditions maintained for *in vitro* culture were 25±2°C, 8 hrs photo period with 2000 lux fluorescent light and 70% relative humidity.

Results and Discussion

I) Shooting

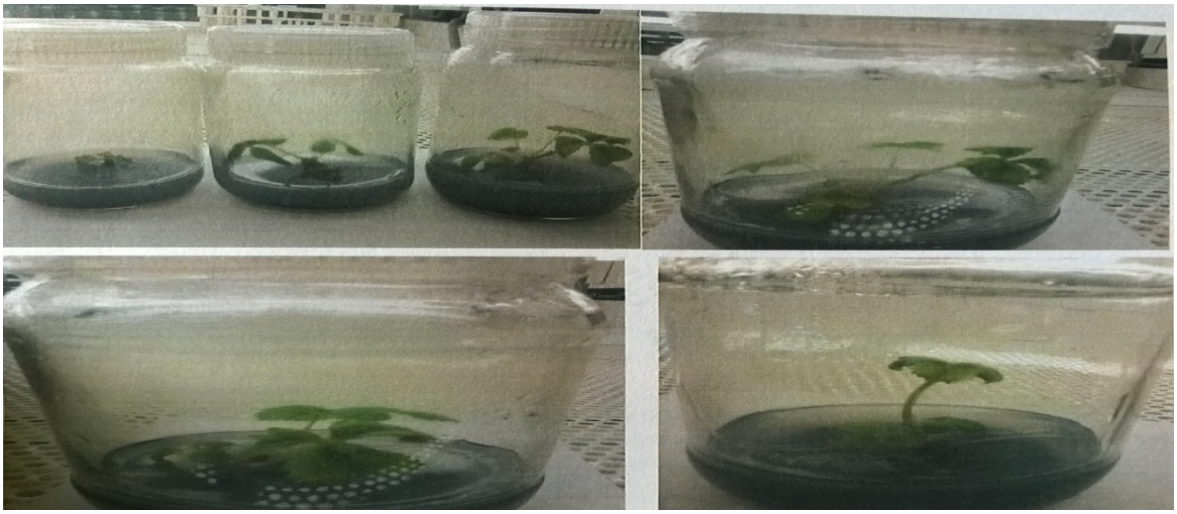
Meristem tip explants was placed ion MS media supplemented with different concentration of combination of BA and KIN Phytohormone culture bottle, together 25±2°C, 70% humidity along hrs light period for three weeks.

Plate 1: Shoot Proliferation of Strawberry in three weeks -

II) Multiplication

Derived plantlets were multiplied by sequential sub culturing into fresh medium in culture bottle, supplemented with 1.5 mg/l BA and 0.5 mg/l KIN hormone and close with plastic caps. At this stage of culture, the shoots were proliferated rapidly within 18-22 days.

Plate 2: Well shooted explants were multiplied



III) Rooting

Shootlets of strawberry obtained from multiplication stage were cultured on different concentration of IBA. After 4 weeks, data were recorded.

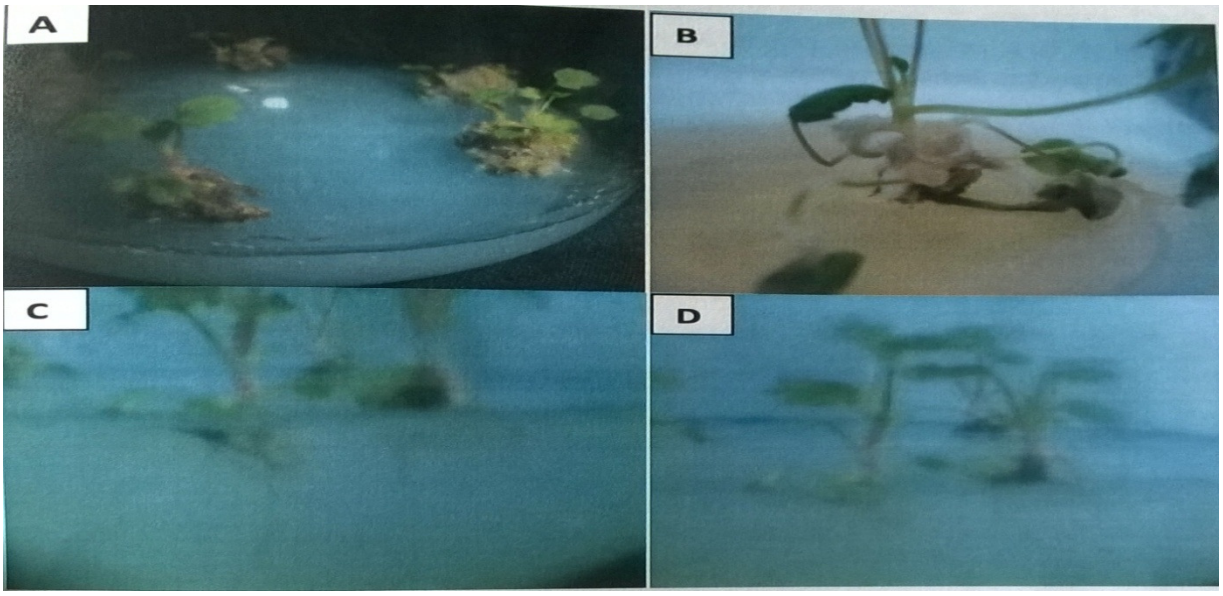


Plate 3: (A) Micro shoot (4-5 cm) inoculated on Rooting medium (B)Roots Developed on Conc. 0.5 mg/l of IBA (C) Roots Developed on conc. 1.0 mg/l of IBA (D)Roots developed on conc. !.5 mg/l of IBA

During micropropagation of Strawberry (*Fragaria* x *Ananassa* Duch.) shoot proliferation by using combination of BA, KIN set of conc. As well as using different conc. Of IBA for root proliferation are showen in plate 1,2,3 respectively.

Treatment of hormones for shooting and rooting gave following result within 3 weeks.

Table 3: Effect of different conc. of BA with KIN on shoot induction from Meristem tip explants of strawberry after 3 week of inoculation

Sr. no.	Growth regulators Conc.(mg/l) BA+KIN	% of explants showing shoot proliferation	Day to shoot Formation
1.	1.4+0.5	60%	18
2.	1.5+0.5	80%	18
3.	2.0+0.1	50%	18
4.	2.0+0.5	60%	18
5.	1.5+0.5 (Charcoal)	90%	18

Culture taken from shooting stage which showed good induction were then used for soot multiplication.

Table 4: Result of multiplication from shooting stage which showed 80 to 90% of Shoot proliferation after 18 to 24 days

Sr. No	Growth regulator Conc.(mg/l) BA+KIN	Day to shoot formation	No of shoot/explants (Mean±SD)
1.	1.5+0.5	16	2.16±0.75
2.	1.5+0.5 (Charcoal)	16	2.83±0.40

Table 5: Effect of different conc. of IBA, plant growth regulator on *in viro* rooing of shoots of strawberry after 4 week of inoculation

Sr No.	Growth regulators conc.(mg/l)IBA	Day to root formation	No of root micro Cutting/S.D
1.	0.5	15	3.2±0.44
2.	1.0	15	3.5±0.54
3.	1.5	15	2.8±1.47

References

Bilgrami, A.K. pandey., 1994 and John H. Dodds, Lorin W, Robert., 1995

Biotechnology-Expanding horizons by B.D Singh., 2003. Kalyani publication. New Delhi.

Biswas,M.Hossian, M.B., Ahmed, U.k, Roy,R,Karim,M,A., Razvy, M.Salahin and R.

Islam. 2007. Multiple shoots regeneration of strawberry under various colour illuminations.., *American-Eurasian Journal of Scientific Reserch2.(2):*133-133.

Boxus. P.H. 1974. The production of strawberry plants by *in vitro* Micropropagation.J. Hortic. Sci. (49), 209-210

Boxus et al., (1989) Review on strawberry mass propagation. Acta. Hort. 265;309-320.

Chawla, H.S., 2002. Introduction to Plant Biotecnology. Science Publisher, Inc., Enfield, New Hampshire.

Debnath et al., (2003) micropropagation of small fruits. In: Jain SM, Ishii K (Eds) Micropropagation of woody trees and fruits, Kluwer Academic Publishers, Dordrecht,Germany, pp 465-506.

Emad Y. Youssief. *In vitro,* propagation of strawberry (*fragaria* x *ananassa* Duch.) Though Organogenesis via Runner Tips. Copy Right © by Emad Y. Youssief2009

Emarah, 2008- Factor affecting propagation of strawberry (*fragaria spp.*) through Tissue culture. J.Praduct. & Dev., 13 (1). 191-212

Fatemeh Haddadi and Maheran Add Aziz, Ghizan Saleh, Azmi Abd Rashid and Hossein Kamaladini. Micropropagation of strawberry cv. Camarosa: Prolific Shoot Regeneration In Vitro Shoot Tip Using Thidiazuron With N6-benzylamino-purine. HORTSCIENCE 45(3):453-456.2010.

Hamish A. Collin, S. Edward., 1998 And George, E., 1993 Plant cell culture, Bios Scientific- publisher, UK, ppl-9

George et al. (eds.).2008. Plant Propagation by tissue Culture 3rd Edition, 465.477.465. Springer

Harrison, E.E, JJ. Luby. G.R. Furnire, and J.F. Hancock., 1997. Micropropagation and molecular variation amongpopulation of octoploid *Fragaria verginiana* and *F chiloensis (Rosaceae)* from North America. Am. J Botany, 84:612-620

Hazarika, 2003- Acclimatization of tissue –culture plants, current science. 85:12-25.

Hu.C.Y.. and P.J Wang, 1983. Meristem shoot tip and bud culture. *In:* Evans DA,